Research Articles

Postmortem Macroscopic Changes in Rats under Rat House Conditions

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Abstract

This research aims to study the postmortem macroscopic changes in rat under rat house conditions. The results of this study will help researchers or veterinarians determine causes of animal death in colonized animals and research studies. This study used thirty-six healthy rats with body weights from 190 to 230 g. Throughout this study, the Animals were housed under controlled environmental conditions of the National Laboratory Animal Center, Mahidol University (22 ± 3 °C, 30 - 70% relative humidity). After euthanasia, animals were maintained in the ventral recumbency position in their cages. Macroscopic changes in the laboratory rat carcasses were observed. Ocular changes were evaluated at 0, 1, 2, 4, 6, 8, 12, 18, 24, 36, and 48 h postmortem. Gross appearances were investigated at 4, 8, 12, 24, and 48 h postmortem in accordance with necropsy examination of internal organs. The results showed that rat carcasses had different characteristics at different times. Ocular changes began immediately 0-4 h after euthanasia, and progressed to total sunken eyes at 24 h. Skin discoloration appeared 8 h after death. Internal findings from livor mortis observations of the internal organs showed that lividity varied from red to purple and became darker with the increasing postmortem interval, especially in the liver and spleen. Moreover, the decomposition of internal organs disintegrated at different rates. The pancreas, gastrointestinal tract, liver, and thyroid showed autolysis early in the postmortem interval (PMI) and increased their severity with time. At 48 h, necropsy revealed that the internal organs were vigorously twisted, collapsed, softened, liquefied, and dissolved. Our results suggest that postmortem ocular changes and macroscopic changes are useful for differentiating postmortem changes from antemortem and can aid in PMI estimation in laboratory rats, which will be beneficial for death investigations concerning laboratory rats.

Keywords: Postmortem changes, Postmortem interval, Postmortem macroscopic changes, Ocular changes

การเปลี่ยนแปลงทางพยาธิสภาพระดับมหกายวิภาคภายหลังการตายของหนูแรทภายใต้สภาพแวดล้อมห้องเลี้ยงสัตว์ทดลอง

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บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาการเปลี่ยนแปลงทางพยาธิสภาพระดับมหกายวิภาคภายหลังการตายของหนูแรทภายใต้สภาพแวดล้อมห้องเลี้ยงสัตว์ทดลอง ซึ่งการศึกษานี้จะเป็นประโยชน์แก่สัตวแพทย์และนักวิจัย ในการผ่าชันสูตรซากของหนูแรทในโคโลนีหรืองานวิจัย เพื่อช่วยในการวินิจฉัยหาสาเหตุการตายของสัตว์ การศึกษานี้ใช้หนูแรทสุขภาพดี น้ำหนัก 190-230 กรัม จำนวน 36 ตัว สัตว์แต่ละตัวถูกแยกเลี้ยงเดี่ยวในกรงภายใต้ห้องเลี้ยงสัตว์ทดลองของศูนย์สัตว์ทดลองแห่งชาติ มหาวิทยาลัยมหิดล ที่มีการควบคุมสภาพแวดล้อม หลังจากการุณยฆาตนำสัตว์ไปไว้ในกรงเดิมในท่านอนคว่ำ (ventral recumbency) การศึกษานี้มุ่งเน้นศึกษาในเรื่องการเปลี่ยนแปลงทางพยาธิสภาพระดับมหกายวิภาคของหนูแรท ประเมิน ocular changes ที่ 0, 1, 2, 4, 6, 8, 12, 18, 24, 36 และ 48 ชั่วโมงภายหลังการตาย และการเปลี่ยนแปลงทางพยาธิสภาพจากการสังเกตและการผ่าชันสูตรซากอวัยวะภายใน ที่ 4, 8, 12, 24 และ 48 ชั่วโมงภายหลังการตาย จากการศึกษาแสดงให้เห็นว่าซากมีการเปลี่ยนแปลงแตกต่างกันในแต่ละช่วงเวลาที่ต่างกัน การเปลี่ยนแปลงของตาพบได้ที่ชั่วโมงที่ 0-4 ต่อเนื่องไปจนพบ sunken eyes ที่ 24 ชั่วโมงภายหลังการตาย การเปลี่ยนแปลงภายนอกร่างกายสัตว์เริ่มพบ skin discoloration ที่ 8 ชั่วโมงภายหลังการตาย ส่วนการเปลี่ยนแปลงภายในร่างกายสัตว์จากการเกิด livor mortis ของอวัยวะภายในแสดงให้เห็นระดับ lividity สีแดงถึงม่วง และเข้มขึ้นตามเวลาที่เพิ่มขึ้นโดยแตกต่างกันในสัตว์แต่ละตัว ซึ่งจะสามารถเห็นได้ชัดเจนในตับและม้าม สำหรับ decomposition ของอวัยวะภายใน พบในระดับที่แตกต่างกันไป ผลการศึกษานี้พบว่า ตับอ่อน ระบบทางเดินอาหาร ตับ และไทรอยด์ จะพบการเกิด autolysis เป็นอวัยวะแรกๆ และมีระดับที่รุนแรงขึ้นตามเวลาที่เพิ่มขึ้น การผ่าชันสูตรซากในชั่วโมงที่ 48 ภายหลังการตาย พบว่าอวัยวะภายในเกิดการ twisted, collapsed, soften, liquefied และ dissolution อย่างรุนแรง การศึกษาส่งผลให้เห็นว่า postmortem ocular change และ macroscopic change สามารถนำมาใช้เป็นข้อมูลพื้นฐานเพื่อเปรียบเทียบแยกแยะระหว่าง postmortem และ antemortem อีกทั้งยังช่วยในการประมาณหรือเทียบเคียงหาระยะเวลาการตายของหนูแรทได้ ซึ่งจะเป็นประโยชน์ในการช่วยวิเคราะห์หาสาเหตุการตายของหนูแรทในห้องเลี้ยงสัตว์ทดลองได้

คำสำคัญ : การเปลี่ยนแปลงภายหลังการตาย การประมาณระยะเวลาการตาย การเปลี่ยนแปลงพยาธิสภาพระดับมหกายวิภาค การเปลี่ยนแปลงของตาภายหลังการตาย

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Introduction

Immediately after death, postmortem changes will begin to develop. These changes include algor mortis, rigor mortis, livor mortis and decomposition (Merck 2013). Understanding general postmortem changes can help to indicate changes of body before (antemortem) and after death. Therefore, estimating the postmortem interval (PMI) is important because it contributes to determining the time frame during which the death occurred. Moreover, postmortem change studies and PMI analysis are a tools used to investigate the cause of death in humans, animals, and including laboratory animals.

Postmortem changes that occur after death results from complex physicochemical and environmental processes. These are affected by factors within the internal body and external factors. Internal factors affect postmortem changes, primarily including the body's surface area, body mass, body fat, open injuries, sepsis, and infection. While external factors hasten the rate of postmortem changes, such as the environment of the death scene and the storage of the body after death (Megyesi et al., 2005; Sutherland et al., 2013; Lowe et al., 2013).

Considerable research has been done to establish reliable and accurate time of death estimation methods in humans. Therefore, the estimated time of death can be used in law enforcement to solve death-related criminal cases. Since there is a significant amount of information about postmortem changes in humans, most animal studies have applied from human forensic. In addition, contemporary veterinary forensic medicine emphasizes PMI refining as one of the key challenges (Munro and Munro 2013).

In laboratory animals, it is necessary to determine or predict the cause of death. Such data would be helpful for research analysis and disease investigations in animal colonies. Furthermore, it is imperative to learn about postmortem macroscopic changes as it can help to differentiate postmortem from antemortem changes. Similarly, numerous methods have been proposed for the determination of PMI. Literature relating to early PMI estimation has continually adapted techniques, mainly focusing on algor mortis and rigor mortis. In rodents, the study by Dwi et al. (2017) showed an algor mortis pattern of rats at 0 to 22 h under ambient room and condition room. In addition, published data exist regarding the process of evolution and resolution of rigor mortis in rats, rigor was evaluated for each joint in time points (Krompecher 2016).

A macroscopic change is one of the essential tools. Morphologic changes begin immediately after death and continue over a prolonged period at different rates in different organs. Generally, visible changes on a gross level consist of discoloration, livor mortis, a sequence of decomposition (Brooks 2016) etc. For postmortem gross change, limited information is available on wildlife and companion animal species. One studies by Erlandsson and Munro (2007) on beagles showed the external and internal gross pathology observation at various time points after death. Some authors refer to the forensics of livestock, but studies on laboratory animals are limited or lacking in terms of data.

Nonetheless, a few studies have evaluated postmortem ocular change by macroscopic examination methods, such as a study on postmortem eye changes on corneal opacity and eye sinking in rodents to estimate the PMI (Capas-Peneda et al., 2016). Even though countless studies have investigated human postmortem changes and forensics, only a few of research have been conducted concerning postmortem changes and estimation of PMI in animals, especially laboratory animals. Understanding

postmortem changes corresponding to their PMI in laboratory animals can help researchers or veterinarians determine the cause of animal death in colonized animals and research studies. Thus, the objectives of this study is to evaluate postmortem changes at different death intervals. The observations included ocular, gross appearances, and internal organs. The results will be collected and developed as database of general postmortem changes, plus to estimate PMI in the laboratory animal house facility.

Materials and Methods

Animal

The study protocol was approved by National Laboratory Animal Center Animal Care and Use Committee (protocol number RA2021-17).

This study was carried out on 36 healthy Rattus norvegicus (Wistar rat). The Rats were divided into postmortem ocular change evaluation and gross change evaluation. The Animals were obtained from the Animal Production Office of National Laboratory Animal Center, Mahidol University. The animals were quarantined for a day and housed individually in open-topped cages with stainless steel wire-bar and solid bottom. Physical enrichment was provided while the rats were individually housed, including ribbon water hyacinth and PVC pipe. The cages contained mixed bedding of corn cobs and water hyacinth. Food and hyper chlorinated water (5-7 ppm.) were provided ad libitum. The housing conditions were controlled at a temperature of 22 ± 3 ^{o}C, with relative humidity (30-70 %) and ventilation rate >10 ACH, respectively, according to the Guide for the Care and Use of Laboratory Animals, Eight Edition. The animals were exposed to 12:12 hours of a light-dark cycle during this study. For the euthanasia method, rats were administrated 250 mg/kg of thiopental sodium intraperitoneally. After death was confirmed, the carcass was raised in ventral recumbency in their cages.

Postmortem ocular change evaluation

Sixteen rats (8 of each gender) were used in this study; their body weight ranged from 200 to 230 g. After the animals were euthanized, ocular changes were evaluated by macroscopic examination at 0, 1, 2, 4, 6, 8, 12, 18, 24, 36, and 48 h. The eyes were observed and given a score from 0 to 4, according to Table 1. The data were reported as the sample mean ± standard deviation (mean ± SD)

Table 1. Classification for postmortem ocular changes (Capas-Peneda et al., 2016).

Score	Ocular changes
0	Normal
1	Mild Corneal Opacity
2	Total Corneal Opacity
3	Mild Eye Sinking
4	Total Eye Sinking

Postmortem gross change evaluation

Twenty rats (10 of each gender) were used; the animals' body weight range from 190 - 230 g. Gross appearances were evaluated by observing general changes in the eyes and skin. Moreover, a necropsy was performed to study livor mortis, decomposition, displacement, and alterations of the internal organs. The animals were evaluated at 4, 8, 12, 24, and 48 h (four animals per time).

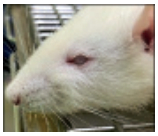
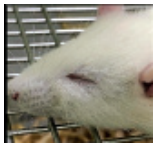
Results

Under the laboratory animal room conditions with normal temperature and relative humidity for the rat house facility, the results indicated that macroscopic changes had occurred at each time point and showed similar appearances in every animal within the same group; the discussion is given below.

Postmortem ocular changes

Ocular mucous membrane changed from pale to purple approximately 0 - 4 h after euthanasia. Mild corneal opacity began at 4 h postmortem observations, and complete total corneal opacity started at 6 h postmortem in some rats. The mild sinking of eyeball occurred during 12 - 18 h in all animals and evolved to total sinking about 24 h postmortem (Table 2).

Table 2. Classification for postmortem ocular changes in rats (n=16).

Ocular changes score		Time postmortem (h)
0 Normal		0 - 2
1 Mild Corneal Opacity		Start 4
2 Total Corneal Opacity		Start 6
3 Mild Eye Sinking		12 - 18
4 Total Eye Sinking		Start 24

Postmortem gross changes

External findings

Discoloration or color change of the skin and soft tissue was found in all animals of the same group. This study found dark red discoloration in the skin, nails, scrotum skin, and gums at approximately 12 h postmortem. The tip of the tongue became darkened, dry, and hard in appearance after 48 h postmortem. Eight hours after death, the caudal abdomen developed dark red to green discoloration. Within 12 h postmortem, dark green/purple discoloration was observed in all rats (Figure 2).

Internal findings

Livor mortis of the internal organs first appeared approximately 4 to 8 h postmortem. Lungs appeared dark purple area at 4 h, and diffuse congestion in all lobes was revealed at 12 h postmortem, while severe congestion was seen in all animals after 48 h (Figure 3). The thymus had mild congestion (reddish-purple spots) at the 8 h necropsy time, and severe congestion was found within 12 to 24 h (Figure 4). The liver and spleen showed dark reddish discoloration, which started to appear at 8 h. These changes were presented in the lower portion of the abdominal quadrants due to hypostatic congestion, as shown in Figure 5. Lividity increased in size and spread all over the dependent regions, and intensified at around 12 to 24 h. The lividity of each animal varied from red to purple and became darkened as the postmortem interval increased.

Decomposition or disintegration of the internal organs also occurred at different rates. The pancreas, gastrointestinal tract, livers, and thyroid showed marked autolysis early in PMI. At 8 to 12 h postmortem, the pancreas and its surrounding fat were soft and reddish, and appearances were similar in all animals (Figure 6A, B). Stomach and gastric mucosa began to soften and lost mucosal folds or rugae. The intestinal lumen was filled with gas, especially in the cecum, and distention and displacement of abdominal organs were found, as shown in Figures 8A, B. The results suggest that bacteria fermentation and gas formation could cause the organ to change position. The thyroid glands were pink to red in color. Moreover, the surface tissue collapsed, softened, and became flabby. Finally, the observation of the livers during these hours showed that the organs became darkened, shrinken, and spongy.

After 24 - 48 hours of PMI, severe autolysis and dissolution of most abdominal organs could be seen at this stage. The pancreas became more softened and liquefied, with tissue breakdown observed (Figure 6C). The results from 48 h postmortem revealed a thin gastric wall and reddish external surface in the stomach portion, which contacted the liver. The gastric mucosa was soft, liquid-like, and there were no rugae in the glandular stomach (Figure 7). The serosal surface of small intestine, large intestine, and mesenteries showed reddish coloration and a thin intestinal wall. A torsion or twist was also found at random parts of the intestinal tract (especially the part of jejunum and ileum) in 3 animals of the 48 h group, as shown in Figure 8D. Some areas of the livers were found to be pale. Another important aspect of this stage was the collapse and softness of many organs. 24 h postmortem, the internal organs including thyroid, livers, spleen, and kidneys were soft, flabby, and congested, as observed macroscopically (Figure 9). Then, 48 h postmortem, the organs of all animals showed an increasing degree of softening and more collapse; the kidneys presented a loss of normal architecture (Figure 10). Moreover, the liquefaction of abdominal fat was also present.

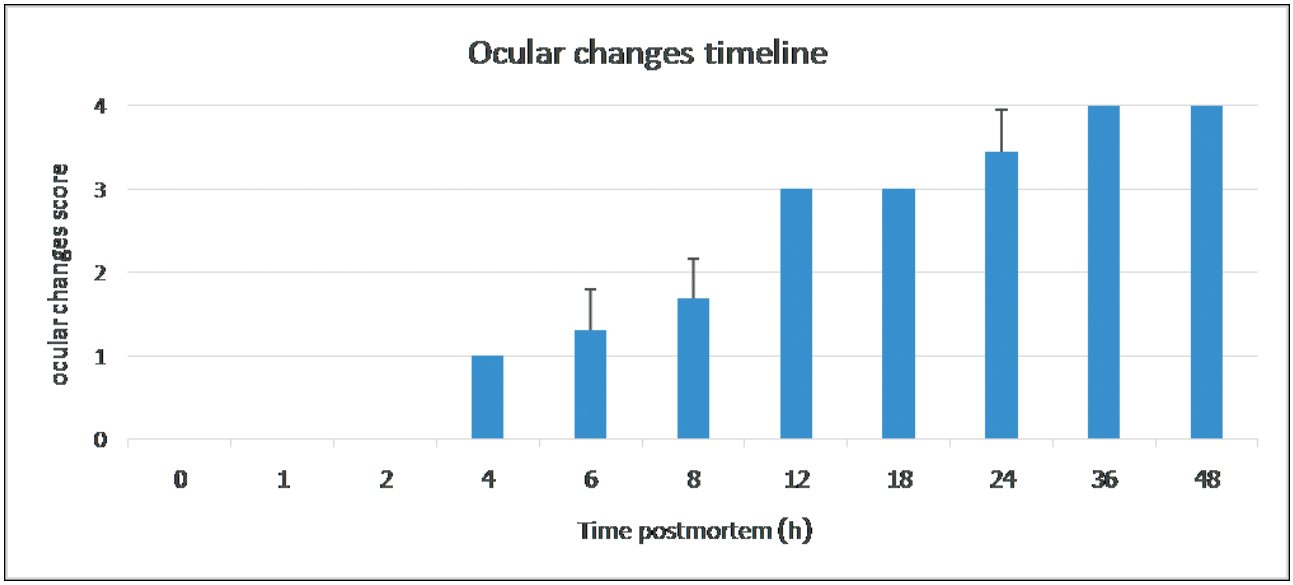


Figure 1. Timeline of postmortem ocular changes in rats (Mean ± SD, n=16).

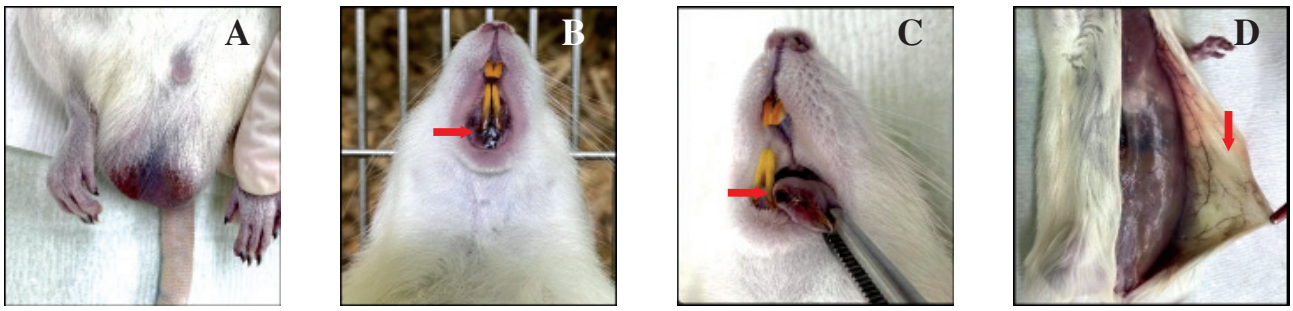


Figure 2. Discoloration of A) the skin on the scrotum and nails shown as dark red, B) gums shown as dark red, C) the tip of the tongue revealed as dark red in appearance, and D) abdominal skin shown as green discoloration.

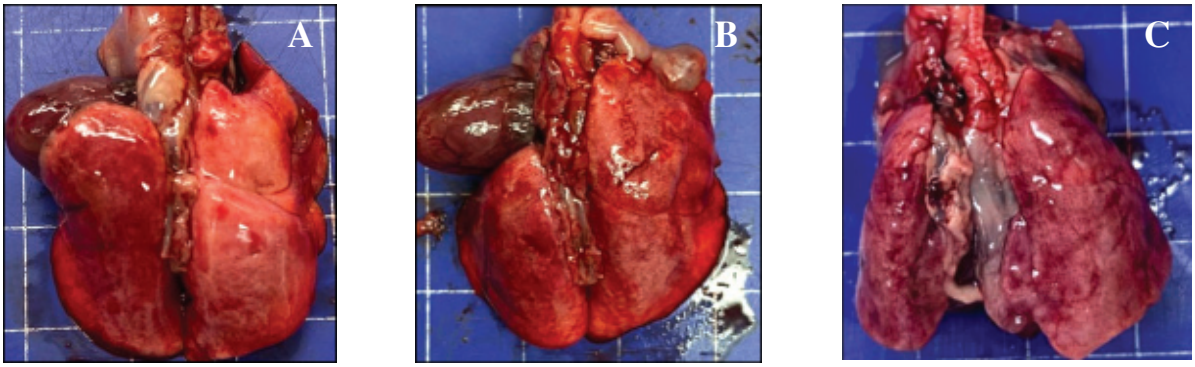


Figure 3. Livor mortis of the lung at A) 4 h PMI, with lungs exhibiting a dark purple area and congestion, B) 12 h PMI, exhibiting diffuse congestion in all lobes, and C) 48 h PMI, showing severe congestion and shrinkage.

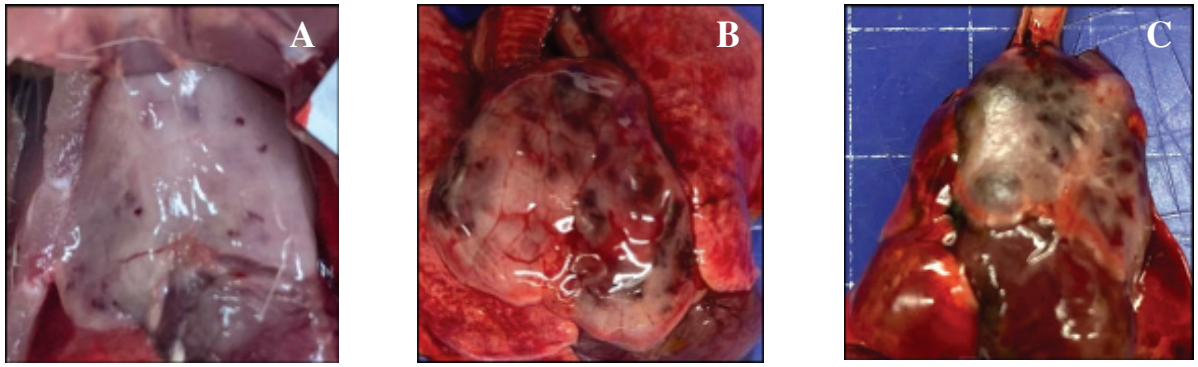


Figure 4. Livor mortis of the thymus at A) 8 h PMI, revealing reddish-purple spots, B) 12 h PMI, showing severe congestion, and C) 24 h PMI, also showing severe congestion.

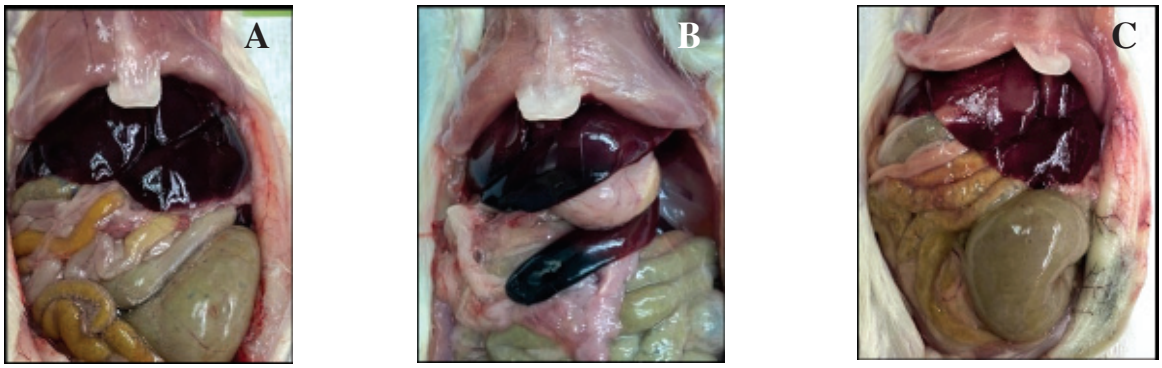


Figure 5. Livor mortis of the liver and spleen at A) 4 h PMI, with no apparent changes were found, B) 8 h PMI, with the liver and spleen showing reddish-blue staining of the low-lying dependent regions of the body, and C) 24 h PMI, revealing an increase in the extent of lividity.

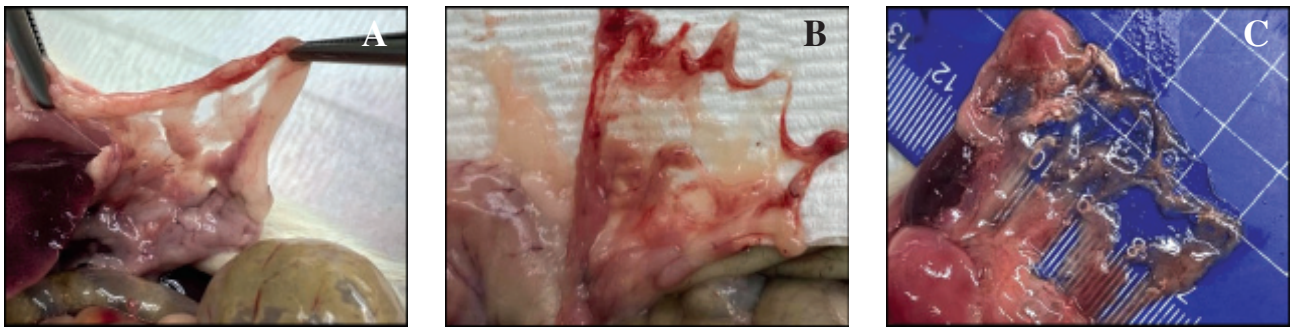


Figure 6. Decomposition changes in the pancreas at A) 4 h PMI, showing soft tissue. B) 12 h PMI, showing soft, and red coloration, and C) 48 h PMI, revealing an increased level of softening, almost liquefied, and breakdown of tissue.

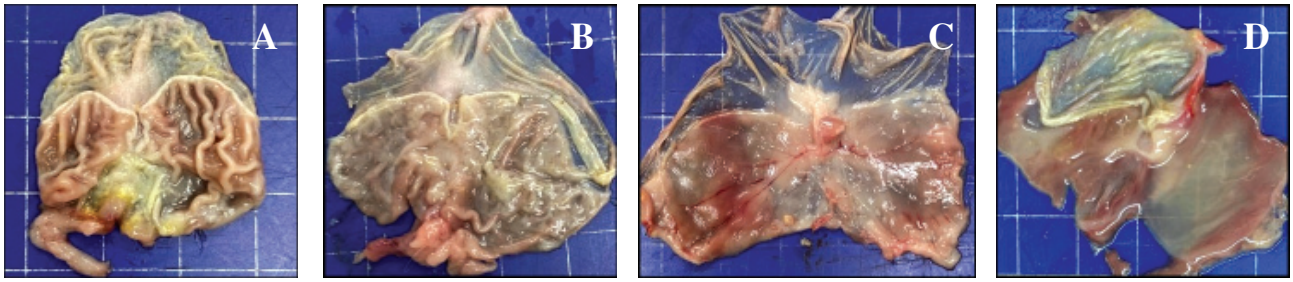


Figure 7. Gastric mucosal surface at A) 4 h PMI, with no apparent changes found, B) 12 h PMI, which found gastric mucosa beginning to soften and less mucosal folds or rugae, C) 24 h PMI, which revealed a thin gastric wall and a red stain on the external surface, while gastric mucosa was soft and rugae was lost, and D) 48 h PMI, were almost liquid.

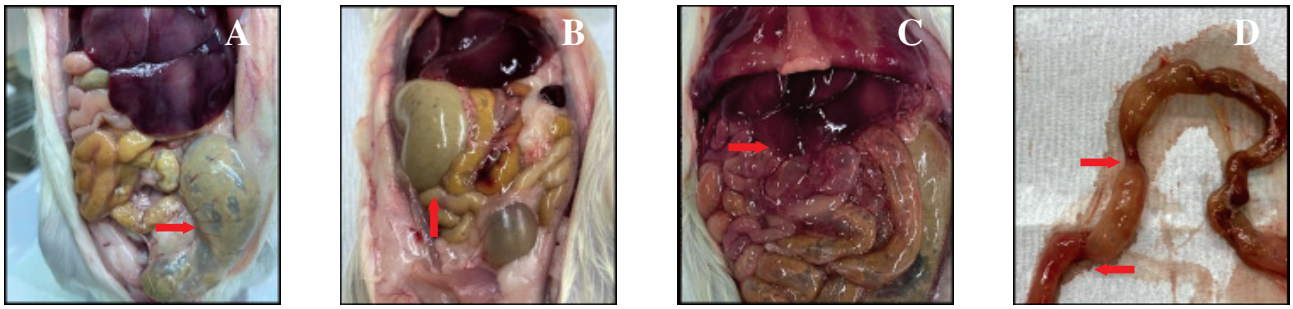


Figure 8. A, B) Intestinal distension and displacement causing changes in the position of organs (8 to 12 h PMI). C) Pinkish- red stain stain on the serosal surface of intestines and thin intestinal wall observed (12 to 48 h PMI). D) Intestinal torsion (48 h PMI).

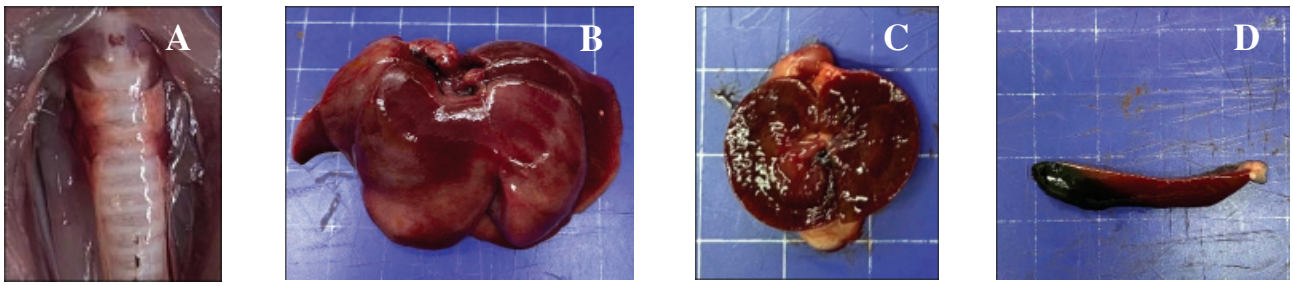


Figure 9. Decomposition changed in 24 h PMI. A) Thyroid were soft, collapsed and congested. B) Livers were soft and congested. C) Kidneys were soft. D) The spleen presented as congested and flabby.

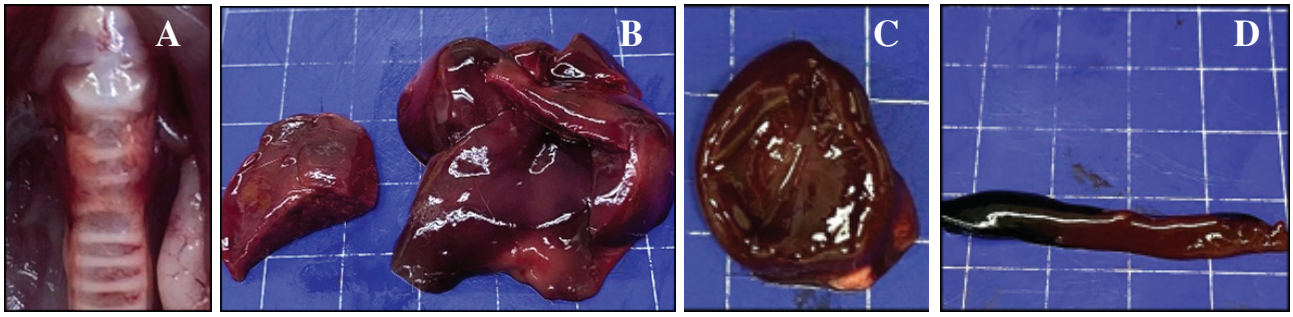


Figure 10. Decomposition changed in 48 h PMI. A) Thyroid presented as more collapsed, soft and flabby. B) Livers were soft, congested, and collapsed. C) Kidney were also soft, flabby and had lost of normal architecture (the cortex and medulla of the kidneys were difficult to distinguish). D) The spleen presented as soft, flabby, and congested.

Discussion

The study focused on the macroscopic changes in laboratory rat carcasses. The experiment was designed to prove the basic concepts of gross findings and related timelines of PMI in rats under laboratory animal house conditions. Interestingly, various changes in rat carcass were found at different times.

In the first examination, ocular changes during the early postmortem period included corneal opacity and sunken eyes. The results found that ocular changes in rat began with clouding at 4 h and evolved to complete total opacity after 6 h postmortem. The total sinking of the eyeballs occurred about 24 h postmortem. The results from this study are similar to data published by Capas-Peneda et al. (2016), in which rats showed total corneal opacification presented at 5 h postmortem, and total eye sinking was evidenced for nearly 20 h. In mice, corneal opacification was completed within the first-hour postmortem, and total eye sinking occurred approximately 19 h postmortem (Capas-Peneda et al., 2016). The results of eye changes are difficult to compare with other animal and human because it depends on whether the eyelids are open or closed. Many studies have also found that pattern changes in the eyes are associated with PMI, such as corneal opacity, pupillary diameter, retinal vessel striation, retinal color changes, and intraocular pressure (Kumar et al., 2012; Salam et al., 2012). Among these patterns, intraocular pressure and corneal opacity were the most significantly related to PMI (Balci et al., 2010; Brook 2016).

The earliest postmortem changes occurred while the body was still in the fresh stage before the breakdown of soft tissues, including rigor mortis and livor mortis. These are of forensic significance (Poloz and O'Day 2009).

Livor mortis (lividity or postmortem hypostasis) is the settling of blood to the downside of an animal body and the gravitational settling of blood and body fluids. This phenomenon results in darker reddish coloration on the downside of the carcass. In this study, livor mortis was apparent as discoloration presented in the mucous membranes (lips, gums, around the eyes) and nails. At the early stages of livor development, the color was red due to the oxygenation of red blood cells. It became darker over time as oxygen levels declined or oxygen dissociation continued after death, resulting in a darker or reddish-purple color (Pounder 2011). Livor mortis could also be found on the internal body surfaces and internal organ, most noticeably on the surface of the lungs. Vascular congestion of the lungs is considered one of lividities. The congestion of blood vessels in the lung is derived from an excess of normal blood and fluid settling or hypostasis which occurs spontaneously in the postmortem period (Perper 2006).

This study found that lividity could be detected as early as 4 - 8 h and was found in the lung, thymus, liver, and spleen. Subsequently, it intensified at around 12 to 24 hours. These results make it difficult to compare times with other rodents or animals because studies on animals comprise limited information. In a human study, livor mortis can be found at several positions, such as externally in the skin and mucous membranes and internally in the abdominal or thoracic viscera. It typically developed within 30 minutes to 2 h (Di Maio and Di Maio 2001) and is fully developed at approximately 10 to 12 h after death (Lew 2005). However, lividity of the internal organs could be misinterpreted as congestion or injury (Perper 2006). In humans, the rate of livor mortis was found to either increasing with accelerated decomposition or decrease with cool ambient temperatures,

constant by 24 to 36 h (DiMaio and DiMaio 2001). However, the appearance of livor differs markedly from case to case due to the environment, location, species, body size, discoloration pattern, and the events surrounding death.

Two mechanisms of autolysis and putrefaction are involved in the process of decomposition. Although decomposition occurs immediately after death through autolysis, macroscopic changes only become notable later when putrefaction has occurred (Cockle and Bell 2017). Autolysis is tissue softening and liquefaction caused by the release of intracellular enzymes, resulting in the breakdown of tissue and organs. Organs containing higher enzymes, such as the pancreas and liver, are autolyzed more rapidly (Merck 2013). From the dissection data in this study, the pancreas was the first tissue that appeared to show autolysis. Next, the changing of the liver was seen. These appearance could be found early in 8-12 PMI.

Putrefaction is the decomposition of the body by the action of microorganisms. After cessation of homeostasis, the body's normal flora migrates from the intestinal lumens to the blood vessels and spreads throughout the body (Elliott et al., 2004). The earliest decomposition change occurs because of the degradation of hemoglobin and the formation of hydrogen sulfide (H_2S) within vessels and tissues by the anaerobic process of bacteria. The reaction of both compounds produced green coloration in the lower abdomen (Amendt et al., 2004). In addition, this study observed the greenish discoloration of the skin over the abdomen, which was the first external sign of putrefaction due to the process previously described above.

In our results, the dark green/purple discoloration of the abdomens of rats was found within 12 h postmortem. In a dog study, greenish discoloration could be found 24 h postmortem (Erlandsson and Munro 2007). The quantity of gas was directly proportional to the increasing bacterial activity. Hydrogen sulfide causing the distension of the abdomen and purging putrefactive liquids from the mouth and nostrils was reported in human autopsy (Lee Goff 2009). Bloating is caused by putrefactive gases, H_2S, being trapped within the body. These gases cause distension of the abdominal organs. As a result, displacement will be found in the abdominal organs (Simmons and Cross 2013). This study found intestinal displacement within 8 to 12 h PMI and torsion of the intestines, even breaking part of the tract at 48 h postmortem. The liver also revealed paleness in different areas due to the pressure exerted by the distended gastrointestinal tract. The time found in this study is probably different from humans and other animals. One study showed that distention is typically most notable in the abdomen and often develops at approximately 60 to 72 h in humans. Meanwhile, abdominal distention may occur with remarkable rapidity in ruminants, this process may occur significantly faster or slower depending on the environmental conditions (Munro and Munro 2008).

Morphological changes were observed in the gastrointestinal tract, from necropsy at 12 h. The results showed pinkish to reddish stains on the outer surfaces of stomach, intestine, and surrounding fat. This is known as hemoglobin imbibition, which is caused by the lysis of red blood cells after autolysis and putrefaction. Moreover, all organs found putrefaction started to appear within 8 to 12 h after death and fully developed within 24 to 48 h. The main changes found included discoloration, softening, and liquefaction. Different from human data,

putrefaction began within an hour of death and the highest microbial activity occurred within the 24 h (Shedge et al., 2021). The fermentation process caused softening and liquefaction of the soft tissues by transforming carbohydrates, lipids, and proteins into organic acids and gases (Byard and Tsokos 2013).

The general physical changes, decomposition stages, autolytic activity, and structural alterations in tissue were primarily detected by observation. Postmortem period characteristic changes include livor mortis, discoloration, ocular change, and gross change. These parameters are helpful for the estimation of the time since death or PMI. However, it highly depends on individuals and environmental factors such as temperature, humidity, cause of death, age, body size, initial pathological conditions, etc. (Henssge and Madea 2007).

In conclusion, it is possible to apply different characteristics from general appearances and necropsy data to refine the estimation of the PMI in rats, based on ocular change and gross observation. In this study, the ocular changes, macroscopic changes in the lung, thymus, liver, spleen and the decomposition of abdominal organs proved to be the most useful organs, highlighting the changes that developed during the 48 h timeframe after death. These data could aid in indicating postmortem and antemortem to prevent misinterpretation. Moreover, PMI could be estimated by evaluating the morphological findings. Analysis of PMI degradation is a primary tool for death investigation in the laboratory rats, which benefits researches and veterinarians. Future research is expected to result in better techniques with increased accuracy in PMI estimation and postmortem change studies in several laboratory animals, which will further beneficial veterinary forensic investigations.

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